Effective treatment of allergic airway inflammation with *Helicobacter pylori* immunomodulators requires BATF3-dependent dendritic cells and IL-10

Daniela B. Engler*a,1, Sebastian Reutenb,1, Yolanda van Wijckc, Sabine Urbanb, Andreas Kyburzb, Joachim Maxeine,b, Helen Martinb, Nir Yogevd, Ari Waismand, Markus Gerhardo, Timothy L. Coverf, Christian Taubec, and Anne Müllera,2

*aInstitute of Molecular Cancer Research, University of Zürich, 8057 Zürich, Switzerland; bMedical Clinic III and cInstitute for Molecular Medicine, Johannes Gutenberg University, 55131 Mainz, Germany; cDepartment of Pulmonology, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands; dInstitute of Microbiology, Immunology and Hygiene, Technical University of Munich and German Center for Infection Research, 81675 Munich, Germany; and eDivision of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine and Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN 37232-2358

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The prevalence of allergic asthma and other atopic diseases has reached epidemic proportions in large parts of the developed world. The gradual loss of the human indigenous microbiota has been held responsible for this trend. The bacterial pathogen *Helicobacter pylori* is a constituent of the normal gastric microbiota whose presence has been inversely linked to allergy and asthma in humans and experimental models. Here we show that oral or i.p. tolerization with *H. pylori* extract prevents the airway hyperresponsiveness, bronchoalveolar eosinophilia, pulmonary inflammation, and Th2 cytokine production that are hallmarks of allergen-induced asthma in mice. Asthma protection is not conferred by extracts from other enteropathogens and requires a heat-sensitive *H. pylori* component and the DC-intrinsic production of IL-10. The basic leucine zipper ATF-like 3 (BATF3)-dependent CD103⁺CD11b⁻ dendritic cell lineage is enriched in the lungs of protected mice and strictly required for protection. Two *H. pylori* persistence determinants, the γ-glutamyl-transpeptidase GGT and the vacuolating cytotoxin VacA, are required and sufficient for asthma protection and can be administered in purified form to prevent asthma. In conclusion, we provide preclinical evidence for the concept that the immunomodulatory properties of *H. pylori* can be exploited for tolerization strategies aiming to prevent allergen-induced asthma.

bacterial immunomodulation | allergy and asthma prevention | tolerogenic dendritic cells | bacterial persistence determinants

**T**he prevalence of asthma and other allergic diseases has increased steadily in the course of the second half of the 20th century in both adult and pediatric, developed and developing populations (1). The lack of early childhood infections or microbial exposure due to improved sanitation, and the gradual loss of the indigenous microbiota have alternately been proposed to account for this major public health trend (2, 3). Epidemiological and experimental studies have consistently shown a strong inverse association of chronic infection with the human gastric bacterial pathogen *Helicobacter pylori* with the risk of developing allergic asthma (4–9). Chronic infection with *H. pylori* is less common in allergic individuals presenting with asthma, hay fever, or eczema than in the general population; this is especially true in children and in patients with early-onset disease (4–8). We have reported earlier that experimental infection of C57BL/6 mice with a mouse-colonizing human isolate of *H. pylori* confers robust protection against allergen-induced asthma, with particularly strong protective effects observed upon early-life exposure (9). Asthma protection could be attributed to *H. pylori*-specific tolerogenic reprogramming of dendritic cells in vitro and in vivo and to the induction of highly suppressive regulatory T cells (9, 10). Despite its striking immunomodulatory properties (11) and remarkable inverse link to various allergic diseases, the use of live *H. pylori* as a therapeutic intervention or preventive measure is unattractive due to the well-documented carcinogenic potential of chronic infection with this organism. *H. pylori* induces gastric and duodenal ulcers (12), and is also widely accepted to be the leading cause of gastric adenocarcinoma (13). Here, we have devised a strategy of *H. pylori*-specific tolerization that harnesses the bacteria’s immunomodulatory properties for the prevention of asthma while avoiding the risks associated with live infection and have elucidated several key determinants of asthma protection in both the bacteria and the host.

**Results**

*H. pylori* Whole Cell Extract Protects Against Allergen-Induced Asthma. To assess whether regular administration of *H. pylori* extract protects against allergen-induced asthma and thus recapitulates the effects of live infection, we treated mice with weekly doses of intragastrically administered whole cell extract from age day 7 onwards before subjecting them to ovalbumin sensitization and challenge. Control mice that had received ovalbumin but no *H. pylori* extract developed airway hyperresponsiveness to methacholine (Fig. 1 A and B and Fig. S1 A–D) and bronchoalveolar immune cell infiltration and eosinophilia (Fig. 1 C and D), as well as histologically evident lung inflammation and infiltration.

**Significance**

Allergic asthma represents an increasingly common public health problem. Here, we provide preclinical evidence for the efficacy of active tolerization using *Helicobacter pylori* components as a viable strategy for asthma prevention. We use a mouse model of allergic asthma to show that regular treatment with *H. pylori* extract effectively alleviates all hallmarks of the disease. Successful treatment depends on the regulatory cytokine IL-10 and on basic leucine zipper ATF-like 3 (BATF3)-dependent dendritic cell lineages. *H. pylori* extracts lacking the γ-glutamyl-transpeptidase GGT or the vacuolating cytotoxin VacA fail to protect against asthma; conversely, both factors can be administered in purified form to achieve protection. In conclusion, the immunomodulatory properties of the common infectious agent *H. pylori* can be exploited for therapeutic purposes in an allergy model.


The authors declare no conflict of interest.

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1D.B.E. and S.R. contributed equally to this work.

2To whom correspondence should be addressed. Email: mueller@imcr.uzh.ch.

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goblet cell metaplasia (Fig. 1 E–G). The restimulation of single cell lung preparations with ovalbumin induced the production of high levels of the Th2 cytokines IL-5 and IL-13 (Fig. 1 H and I). In contrast, mice that had received *H. pylori* extract were protected against airway hyperresponsiveness (Fig. 1 A and B and Fig. S1 A–D), and exhibited significantly lower levels of bronchoalveolar and pulmonary inflammation, eosinophilia and goblet cell metaplasia (Fig. 1 C–G). Th2 cytokine production upon allergen restimulation of lung preparations was also reduced (Fig. 1 H and I). The failure of extract-treated mice to develop allergen-induced symptoms of asthma was not due to an impaired primary response to the allergen, as the levels of ovalbumin-specific serum IgE were similar in all sensitized mice (Fig. S1E).

To address the specificity of the observed effects and elucidate key prerequisites of protection, we examined various administration routes and regimens, ages at treatment onset, and extracts from other gastrointestinal pathogens. Interestingly, the systemic (intraperitoneal) administration of *H. pylori* extract was as efficient as the intragastric route at conferring protection against allergen-induced asthma (Fig. S1 F–I). Intragastric treatment was less effective when initiated in adult mice as opposed to neonates, and four consecutive doses of extract administered to young mice before weaning were insufficient to induce full protection (Fig. S1 F–I). Heat-inactivated *H. pylori* extract, as well as identical amounts of extracts generated from cultures of *Escherichia coli* or *Salmonella typhimurium*, failed to confer protection against the examined hallmarks of allergic airway disease (Fig. S1 F–I). In conclusion, the beneficial effects of extract treatment are specific to *H. pylori* and require a heat-sensitive component of the bacteria, and are most pronounced if the treatment is initiated in young mice.

**Successful Tolerization Against Allergen-Induced Asthma Requires IL-10 and IL-18, but Not Regulatory T Cells.** *H. pylori* is known to induce the production of IL-10 in various immune cell compartments (14, 15) and high gastric levels of IL-10 ensure *H. pylori* persistence and promote *H. pylori*-specific immune tolerance (16, 17). We have shown previously that dendritic cells (DCs) play a critical role in immune tolerance to live *H. pylori*; the depletion of CD11c-positive DCs breaks tolerance and promotes clearance of the bacteria (10). To assess whether DCs produce IL-10 not only in response to live infection as shown (10), but also in response to *H. pylori* extract, cultured murine bone marrow-derived (BM) DCs were treated with increasing concentrations of extract. Indeed, BM-DCs produced and secreted large amounts of IL-10, and this was dependent on TLR2 and MyD88 signaling, but independent of TLR4 (Fig. 2A). A clear dose-dependent secretion of IL-10 could also be observed in human blood-derived DCs from six independent donors cultured with *H. pylori* extract (Fig. 2B). To address whether IL-10 is required for asthma protection conferred by extract tolerization or live infection, we administered two doses of IL-10 receptor (IL-10R)-neutralizing antibody during the challenge phase of the protocol to mice that had either received extract from the neonatal period onwards or had been infected as neonates. IL-10 signaling was required for protection against asthma in both scenarios (Fig. 2 C–F). We further examined the effects of extract tolerization and live infection in mice that are deficient for IL-10 production specifically in the CD11c<sup>+</sup> immune cell compartment. Although not entirely resistant to extract treatment or the beneficial effects of live infection, CD11c-Cre<sup>–/–</sup> mice were less well protected than their Cre-negative littermates, i.e., exhibited significantly higher eosinophil counts, lung inflammation and goblet cell metaplasia (Fig. 2 G–J). The overall secretion of IL-10 by allergen-restimulated lung cells was reduced in CD11c-Cre<sup>–/–</sup> mice (Fig. S2A), implying that CD11c<sup>+</sup> cells represent a major source of pulmonary IL-10 in this setting. In summary, we conclude that *H. pylori* extract induces IL-10 production in both murine and human DCs and that IL-10 produced by CD11c<sup>+</sup> DCs/mononuclear phagocytes, in the lungs and/or at other sites, contributes critically to protection.

Having shown previously that DC-derived IL-18 is a critical mediator of *H. pylori*-induced immune tolerance (10), we next examined the effects of *H. pylori* extract and live infection on IL-18R<sup>−/−</sup> mice. IL-18 signaling was absolutely required for the protective effects of live bacteria as well as extract treatment (Fig. S2 B–E), underscoring the tolerance-promoting role of this cytokine in the context of the *H. pylori*/host interaction. To further address whether Tregs were required for extract-mediated protection (as they are for live infection; ref. 9), we depleted CD25<sup>+</sup> Tregs (>90% depletion efficiency in the lungs, Fig. S2F) by applying two doses of a CD25-specific antibody before ovalbumin challenge. Treg depletion had no effect on the protection from allergic asthma conferred by *H. pylori* extract (Fig. S1 G–J); this result was consistent with a lack of protective activity of CD25<sup>+</sup> Tregs that were adoptively transferred from extract-treated mice to naive recipients (Fig. S1 G–J). *H. pylori* extract treatment of BM-DCs, in contrast to live infection, further failed to promote the expression of the Treg lineage-defining transcription factor FoxP3 in cocultured naive T cells (Fig. S2K), suggesting that *H. pylori* extract exerts its protective activity through the DC-intrinsic production of IL-10 (and IL-18), but independently of Tregs.

**BATF3-Dependent DC Lineages Are Required for *H. pylori*-Induced Protection Against Allergic Airway Inflammation.** Having identified DCs as critical mediators of *H. pylori*-specific tolerance (10) and
as key producers of protective IL-10 (Fig. 2), we next sought to dissect the role of specific DC subsets in the context of H. pylori infection and tolerization. To this end, we generated single cell lung preparations from extract-treated, infected, and positive as well as negative control mice and subjected them to quantitative flow cytometric analysis of various lung-infiltrating DC populations. Interestingly, despite the fact that the mice in examined in this fashion exhibited very typical levels of protection (Fig. S3 A–D), their lungs were infiltrated with the same overall numbers of CD11c+ MHCII+ cDCs as the lungs of asthmatic mice (Fig. 3A). However, when we distinguished between conventional and plasmacytoid DCs (cDCs, pDCs) based on their expression of B220 (also known as CD45R, a marker of the B-cell lineage that is also shared by pDCs, Fig. S3E), we found that CD11c+ MHCII+ B220+ cDCs were relatively more abundant in asthmatic mice, whereas CD11c+ MHCII− B220+ pDCs were more abundant in the lungs of protected mice (Fig. 3 B–D). Furthermore, the total numbers of lung-infiltrating pDCs were higher than in nonsensitized negative controls (Fig. S3F), indicating that pDCs are actively recruited to the lungs of allergen-challenged mice that are either infected with H. pylori or treated with H. pylori extract. Another interesting difference was found among asthmatic and protected mice when we discriminated between CD11b+ and CD103+ cDC subsets (Fig. S3G). Strikingly, whereas the asthmatic lungs of positive control mice were predominantly infiltrated by CD11b+ cDCs, the lungs of protected mice were relatively more infiltrated by CD103+ cDCs (Fig. 3 E–G). Again, CD103+ cDCs appeared to be specifically recruited to the lungs of allergen-challenged mice either infected with H. pylori or treated with H. pylori extract (Fig. S3H).

To assess the functional relevance of CD103+ lung-infiltrating DCs in asthma protection in our model, we examined mice lacking the transcription factor basic leucine zipper ATF-like 3 (BATF3), which has previously been shown to direct the development of CD8α+ lymphoid tissue DCs as well as CD103+ CD11b− DCs in the lungs, intestine and skin (18). We were able to confirm that the lungs of BATF3−/− mice are entirely devoid of CD103+ DCs, and exhibit normal and higher frequencies of pDCs and CD11b+ DCs, respectively (Fig. S3 I and J). Interestingly, pure populations of mesenteric lymph node-derived DCs from BATF3−/− mice failed to express IL-10 upon treatment with increasing doses of H. pylori extract ex vivo (Fig. 3H). BATF3−/− mice were significantly less protected than wild-type mice against allergen-induced asthma upon infection with H. pylori, and upon treatment with H. pylori extract (Fig. 3 I–L), despite being colonized at comparable levels (Fig. S3K). In summary, BATF3-dependent CD103+ DC lineages infiltrate the lungs of protected mice, and are required for the H. pylori-driven, IL-10-mediated protection from allergic asthma.

The H. pylori Persistence Determinants γ-Glutamyl Transpeptidase and Vacuolating Cytotoxin Are Required and Sufficient for Protection Against Allergic Airway Inflammation. We have shown recently that two H. pylori virulence determinants encoded by all clinical isolates investigated to date, the γ-glutamyl transpeptidase GGT and the vacuolating cytotoxin VacA, promote tolerance through tolerogenic reprogramming of DCs (19). To examine whether GGT and/or VacA contribute to asthma protection conferred by extract tolerization, we compared the protective properties of extracts from wild-type bacteria and from GGT- or VacA-deficient isogenic mutants. Interestingly, both mutant extracts were consistently less efficient than wild-type extract at protecting allergen-sensitized and -challenged mice against bronchoalveolar and pulmonary inflammation, eosinophilia, and goblet cell metaplasia (Fig. 4 A–D). To examine whether either factor alone is sufficient to provide protection, we intraperitoneally administered either recombinant GGT or oligomeric VacA purified from culture supernatants of H. pylori once weekly from day 7 of age onwards. No adverse effects were observed in any of the mice, despite their young age at the time of the first doses. Strikingly, both VacA and GGT provided a level of protection against asthma that was comparable to the protection conferred by parallel whole cell extract treatment (Fig. 4 E–H). VacA was somewhat more protective than GGT at identical concentrations, VacA lacking an amino-terminal hydrophobic region of three tandem GXXG motifs that is essential for VacA’s cytotoxic activity (20) failed to protect against asthma (Fig. 4 E–H). Wild-type, but not mutant, VacA had similar effects on pulmonary Th2 cytokine production and DC infiltration (Fig. S4 A–E) as live H. pylori or whole cell extract (Figs. 1 and 3). IL-10R neutralization during ovalbumin challenge abrogated the protective activity of VacA, which was observed not only upon intraperitoneal administration, but also upon oral administration, and in adult as well as neonatally treated mice (Fig. S4 F–J). We conclude that GGT and VacA are key determinants of H. pylori-induced asthma protection and may be administered in purified form to prevent allergic asthma.
Discussion

We have devised here a strategy of active tolerization for the prevention of allergic asthma that exploits the immunomodulatory properties of *H. pylori* without exposing to the risks associated with live infection. By orally or intraperitoneally administering *H. pylori* whole cell extract to allergen-sensitized mice, we were able to achieve a level of protection against asthma that was equivalent to the protection conferred by live infection (9). Extract-mediated protection was highly specific to *H. pylori*, i.e., was not conferred by extract from other Gram-negative enteropathogens such as *E. coli* or *Salmonella typhimurium*. The treatment was particularly successful when initiated in young mice, an observation that is in line with the superior protection afforded by experimental (live) infection of neonatal relative to adult mice (9). The differential susceptibility to successful tolerization of neonates and adults may be attributable to the general tolerogenic bias of the immature neonatal immune system, with its higher Treg/Teffector cell ratios and Treg-predominant responses to foreign antigens (21). Our results are in line with the epidemiological finding that children benefit more from harboring *H. pylori* than adults in terms of their asthma risk (5). The data presented here thus imply that children at high risk of developing asthma are more likely than adults to benefit from *H. pylori*-specific tolerization strategies.

Having shown earlier that *H. pylori*-specific immune tolerance is a consequence of tolerogenic reprogramming of DCs by the bacteria (10), we set out to examine the contribution of specific DC lineages and their immunomodulators to immune tolerance and asthma protection in the settings of neonatal infection and neonatal-onset tolerization with *H. pylori* extract. A careful immunophenotypic analysis of the DC subsets infiltrating the lungs of protected mice revealed a preferential recruitment of CD103<sup>+</sup>CD11b<sup>−</sup> conventional DCs, and to a lesser extent of B220<sup>+</sup> plasmacytoid DCs. The contribution of CD103<sup>+</sup> DCs to asthma protection was further functionally assessed in mice lacking the BATF3 transcription factor, which drives the development of CD8α<sup>+</sup> lymphoid tissue-resident DC lineages and of the closely related CD103<sup>+</sup>CD11b<sup>−</sup> DC lineages in various tissues including the lung, intestine and skin (18). We were able to confirm that CD103<sup>+</sup>CD11b<sup>−</sup> DCs are completely absent from the lungs of BATF3<sup>−/−</sup> mice, whereas all other examined subsets are present in normal numbers. BATF3<sup>−/−</sup> animals were equally susceptible to allergen-induced asthma as wild-type mice;
however, neither regular extract treatment nor H. pylori colonization had any detectable beneficial effect on the examined hallmarks of asthma in this strain, indicating that BATF3-dependent DCs are strictly required for protection in both scenarios. We further found that lymph node-derived DCs from BATF3−/− mice fail to produce IL-10 upon treatment with H. pylori extract ex vivo. This observation is well in line with the requirement for IL-10 signaling proficiency and, more specifically, for the DC-intrinsic production of IL-10 for optimal H. pylori-mediated protection against allergic asthma. We conclude from the combined results that BATF3-dependent DC lineages suppress pulmonary allergen-specific immune responses by production of IL-10; in contrast, Tregs are not critically required for H. pylori extract-mediated protection, as their depletion fails to abrogate protection.

Another cytokine known to be induced by H. pylori, IL-18 (22), also turned out to be absolutely essential for asthma prevention in the course of our studies. IL-18 is produced upon inflammatory activation by H. pylori in a variety of cell types, including DCs, and promotes Treg differentiation and H. pylori-specific tolerance in vitro and in vivo (10, 22). Whether the DC-intrinsic production of IL-18 is required for H. pylori (extract)-mediated protection against asthma remains to be addressed with suitable mouse strains.

Our data further show that two H. pylori determinants, GGT and VacA, are required for extract-mediated protection and can be administered in purified form to prevent allergic asthma. These findings are in line with earlier reports showing that both factors have a critical role in H. pylori persistence and immune modulation. Mutants lacking the ggt gene are incapable of colonizing mice persistently (19, 23), and this phenotype has been attributed to DC tolerization by GGT in vitro and in vivo (19). Similarly, a vacA gene deletion mutant fails to tolerize DCs and to induce Tregs in vivo, and is therefore effectively controlled or even cleared upon onset of an adaptive immune response (19). The fact that a mutant form of VacA lacking an amino-terminal hydrophobic region of three tandem GXXXG motifs fails to protect against asthma when administered to mice in purified form suggests that membrane insertion by VacA is required for its immunomodulatory effects. The exact mechanism and relevant target cell types of VacA in vivo remain to be elucidated in detail. Taken together, our data demonstrate, to our knowledge for the first time, that the immunomodulatory properties of a very common infectious agent in humans, H. pylori, can be exploited for therapeutic purposes in an allergy model and lend

![Fig. 4.](image)

**Fig. 4.** H. pylori GGT and VacA are required and sufficient for protection against asthma. (A–D) Groups of mice were treated as described in Fig. 1 with H. pylori extract generated from either wild-type (WT) or Δggt or ΔvacA mutant bacteria, and were subjected to ovalbumin sensitization and challenge. (E–H) Mice were i.p. injected weekly with 25 μg per dose of either recombinant GGT or purified wild-type or mutant (Δ6–27) VacA starting on day 7 of age until the second sensitization. Total cells (A and E) and eosinophils (B and F) contained in 1 mL of BALF. Tissue inflammation (C and G) and goblet cell metaplasia (D and H).
support to *H. pylori*-specific tolerization as a viable strategy for asthma prevention in high-risk individuals.

**Materials and Methods**

**Animal Experimentation.** C57BL/6, BATF3−/−, IL-18R−/−, and CD11c-Cre;IL-10fl/fl mice were orally infected with *H. pylori* PMSS1 as described (16) or received either once-weekly oral or i.p. doses of 200 μg of extract of *H. pylori* wild-type PMSS1, PMSS1ggt or PMSS1vacA (19), Salmonella typhimurium, or E. coli or once-weekly i.p. doses of 25 μg of recombinant GGT, or of s1m1 type VacA (wild type or Δ66–77, ref. 20) purified from *H. pylori* strain 60190. Mice were sensitized by i.p. injection of 20 μg of ovalbumin (Sigma-Aldrich) emulsified in 2.25 mg of aluminum hydroxide (Alum Inject; Pierce) at 8 and 10 wk of age and challenged with 1% aerosolized ovalbumin from an ultrasonic nebulizer (NE-U17; Omron) for 20 min daily on days 31, 32, and 33 after initial sensitization. Airway resistance measurements were performed on anesthetized, intubated and mechanically ventilated mice (FinePointe Resistance and Compliance System, Buxco Electronics) in response to increasing doses of inhaled metacholine. In vivo blocking of IL-10 signaling and depletion of Tregs was achieved by two i.p. injections of 250 μg of anti-IL-10 antibody (clone 181.3A) and anti-CD25 antibody (clone PC-61.5, both BioXCell), respectively, during the challenge phase. CD4+CD25+ Tregs were adoptively transferred as described (9). Lungs were lavaged via the trachea with 1 mL of PBS. Broncho-alveolar lavage fluid (BALF) cells were counted using trypan blue dye exclusion. Differential cell counts of macrophages, lymphocytes, neutrophils, and eosinophils were performed on cytocentrifuged preparations stained with the Microscopy Hemacolor-Set (Merck). For lung histopathology, lungs were fixed by inflation and immersion in 10% (vol/vol) formalin and embedded in paraffin. Tissue sections were stained with H&E and periodic acid–Schiff and ex-MICROBIOLOGY

**Preparation of *H. pylori* Extract and Purification of GGT and VacA.** *H. pylori* was cultured in *Brucella* broth supplemented with 10% FCS, pelleted by centrifugation, and washed once with PBS. Bacteria were subjected to three freeze-thaw cycles and disrupted by three passes through a French pressure cell press (Stansted Fluid Power, Cell Pressure Homogenizer) at 30,000 bar. Cell debris was removed by centrifugation and the supernatant filtered through a 2-μm filter. Protein concentrations were determined by BCA Protein Kit (R&D Systems). *H. pylori* VacA was purified using published procedures (24, 25), with the following slight modifications. *H. pylori* strain 60190 was cultured in sulfite-free *Brucella* broth containing either cholester or 0.5% charcoal. After centrifugation of the culture, supernatant proteins were precipitated with a 50% saturated solution of ammonium sulfate. The oligomeric form of VacA was isolated by gel filtration chromatography with a Superose 6 HR 16/50 column in PBS containing 0.02% sodium azide and 1 mM EDTA. *H. pylori* GGT was purified as described (19). Protocols for lung single cell preparation, flow cytometry, cytokine ELISAs, and preparation of murine and human DCs and DC:T-cell cocultures can be found in SI Materials and Methods.

**Statistics.** All statistical analysis was performed using Graph Pad prism 5.0 software. The Mann–Whitney test was used throughout. *P* values <0.05 were considered significant.

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Supporting Information

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SI Materials and Methods

Lung Single Cell Preparation, Flow Cytometry, and Th2 Cytokine ELISAs. Lungs were dissected, enzymatically digested with 0.5 mg/mL collagenase type IA (Sigma-Aldrich) and pushed through a 70-μm nylon cell strainer. The antibodies used for staining were anti-MHCII (clone M5/114.15.2), anti-B220 (RA3-6B2), anti-CD11c (clone HL3), anti-CD103 (clone M290), and anti-CD11b (clone M1/70; all BD Pharmingen). FACS analyses were performed on a FACSCanto2 cytometer (BD Biosciences); post-acquisition analysis was done using FlowJo software (Tree Star). Cytokines in lung single-cell cultures restimulated for 72 h with 250 μg/mL ovalbumin were quantified by ELISA (IL-5, BD Pharmingen; IL-13, R&D Systems).

Preparation of Murine and Human DCs and IL-10 ELISA. For generation of murine bone-marrow-derived dendritic cells (BM-DCs), bone marrow isolated from the hind legs of donor mice (BL/6.TLR2−/−, BL/6.TLR4−/−, BL/6.MyD88−/− mice, all from Jackson Labs) was seeded at 50,000 cells per well in 96-well plates in RPMI/10% (vol/vol) FCS and 4 ng/mL GM-CSF and cultured for 5 d. For the isolation of MLN-DCs, mesenteric lymph nodes were digested in 1 mg/mL collagenase (Sigma-Aldrich) for 30 min at 37 °C with shaking before filtering through a cell strainer (40 μm; BD Biosciences) and immunomagnetic isolation of DCs using mouse-specific CD11c microbeads (Miltenyi Biotec). BM-DCs and MLN-DCs were stimulated with the indicated amounts of Helicobacter pylori PMSS1 extract for 16 h, and supernatants were subjected to mIL-10 ELISA (BD Pharmingen). Human monocyte-derived dendritic cells were generated from peripheral blood mononuclear cells. Venous blood was drawn from six healthy volunteers according to protocols approved by the Institutional Review Board of Leiden University Medical Center. Cells were collected after density gradient centrifugation on Ficoll, and CD14+ monocytes were positively isolated by magnetic-activated cell sorting (MACS) using CD14 microbeads (Miltenyi Biotec). Cells were cultured in RPMI-1640 (Invitrogen) supplemented with penicillin (100 U/mL, Astellas Pharma), streptomycin (100 μg/mL, Sigma), pyruvate (1 mM, Sigma), glutamate (2 mM, Sigma), 10% FCS, 20 ng/mL human recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF, Invitrogen/Life Technologies), and 0.86 ng/mL human rIL-4 (R&D Systems) for 6 d. On day 3, the medium and the supplements were refreshed. Monocyte-derived DCs were stimulated with H. pylori extract for 48 h. Secretion of IL-10 by the DCs in the supernatant was measured by ELISA (Sanquin).

DC/T-Cell Cocultures. For Treg differentiation ex vivo, BM-DC cultures were infected overnight with wild-type H. pylori PMSS1 or treated with 25 μg/mL H. pylori extract. Bacteria were killed with 200 U penicillin/0.2 mg streptomycin/mL for 6 h before the addition of T cells. CD4+CD25− T cells were prepared from single-cell suspensions of naive C57BL/6 spleens by immunomagnetic sorting (R&D Systems). DCs were cocultured with CD4+CD25− T cells at a ratio of 1:2 (0.5 × 10^5 DC to 1 × 10^5 T cells) in RPMI containing 10% FCS, 10 ng/mL rTGF-β (PeproTech), 10 ng/mL rIL-2 (R&D Systems) and 1 μg/mL anti-CD3ε (BD Bioscience). After 72 h of coculture, the cells were stained first for CD4 and CD25 and then, after fixation and permeabilization, for FoxP3 (FoxP3-APC, eBioscience). The percentage of FoxP3+ CD4+ T cells was assessed by FACS on a Cyan ADP 9 instrument (Beckman Coulter) and analyzed using FlowJo software (TreeStar).

Processing of Gastric Tissue for Plating and Colony Counting. Stomachs were retrieved and dissected longitudinally. For the quantitative assessment of H. pylori colonization, a stomach section containing representative amounts of antral and corpus tissue was homogenized in Brucella broth and serial dilutions were plated on horse blood plates for colony counting as described (1).

The protection against asthma conferred by treatment with whole cell extract is specific to H. pylori, depends on a heat-sensitive component of the bacteria, and is most efficient when initiated in newborn mice. (A–E) Mice were sensitized i.p. with alum-adjuvanted ovalbumin at 8 and 10 wk of age and challenged with aerosolized ovalbumin 2 wk after the second sensitization to induce asthma-like symptoms. Mock-sensitized mice served as negative controls. One group received once-weekly doses of 200 μg of H. pylori extract from day 7 of age until the second sensitization. (A–D) Airway hyperresponsiveness in response to the indicated increasing doses of methacholine. (E) Ovalbumin-specific serum IgE titers of the groups shown in A–D. (F–I) Mice were treated as described in A–E, with the following modifications: Hpneo, extract administered orally from day 7 to the second sensitization, exactly as described above; Hpi.p., extract administered i.p. from day 7 to the second sensitization; Hpi.p., extract administered orally to adult mice for 4 wk before the second sensitization; Hpneo, extract administered four times orally in the first 3 wk of life only; Hpneo., heat-inactivated extract administered orally from day 7 to the second sensitization; Stneo/ad, E. coli extract administered orally either beginning in the neonatal period or to adults, respectively. (F and G) Total cells and eosinophils contained in 1 mL of BALF. (H and I) Tissue inflammation and goblet cell metaplasia. Data points are pooled from two independent studies.
The protection against asthma conferred by *H. pylori* depends on IL-10 and IL-18, but not on regulatory T cells. (A) CD11c-Cre.IL-10^fl/fl^ mice and their IL-10^fl/fl^ littermates were treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge as described in Fig. 2–G–J. IL-10 secretion by single cell lung preparations restimulated with ovalbumin, as assessed by ELISA. (B–E) WT C57BL/6 and IL-18R^-/-^ mice were neonatally infected with *H. pylori* or treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge as described in Fig. 1. (B and C) Total cells and eosinophils contained in 1 mL of BALF. (D and E) Tissue inflammation and goblet cell metaplasia. (F–J) Wild-type C57BL/6 mice were treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge. One group received two doses of anti-CD25 antibody during ovalbumin challenge. Additional sensitized and challenged groups received 100,000 immunomagnetically isolated CD4^-^CD25^-^T cells from either neonatally infected donors or extract-treated donors i.v. 2 d before the first ovalbumin challenge. (F) Lung infiltration of CD4^-^CD25^-^FoxP3^-^T cells as assessed in lung single cell preparations of individual mice. (G and H) Total cells and eosinophils contained in 1 mL of BALF. (I and J) Tissue inflammation and goblet cell metaplasia. (K) BM-DCs were either infected overnight with live *H. pylori* or treated with 25 μg/mL *H. pylori* extract. Bacteria were killed with antibiotics before the addition of splenic CD4^-^CD25^-^T cells at a DC:T-cell ratio of 1:2. DC:T-cell cocultures were supplemented with rTGF-β, rIL-2, and anti-CD3e. After 72 h of coculture, the cells were stained for CD4, CD25, and FoxP3 and the fraction of FoxP3^-^CD4^-^T cells was assessed by FACS.
Fig. S3. CD103+ conventional DCs accumulate in the lungs of *H. pylori*-infected and extract-treated wild-type mice, but not basic leucine zipper ATF-like 3 (BATF3)−/− mice. (A–D) Total cells and eosinophils contained in 1 mL of BALF, as well as tissue inflammation and goblet cell metaplasia as assessed on H&E and PAS-stained tissue sections, of all mice for which lung DC populations are shown in Fig. 3 A–G. (E) Plasmacytoid DCs (pDCs) and conventional DCs (cDCs) differentially express B220; a representative scatter plot is shown for all CD11c+ MHCII+ DCs of an extract-treated mouse. (F) Total numbers of B220+ pDCs infiltrating the lungs of the mice shown in Fig. 3 A–G. (G) Two distinct cDC lineages can be discriminated in the lung based on CD103 and CD11b expression; a representative scatter plot is shown for an extract-treated mouse (gated on CD11c+ MHCII+ B220− DCs). (H) Total CD103+ cDC infiltration into the lungs of the mice shown in Fig. 3 A–G. (I and J) BATF3−/− mice lack CD103+ cDCs, but retain CD11b+ cDCs and normal frequencies of pDCs. (K) *H. pylori* colonization of neonatally infected WT C57BL/6 and BATF3−/− mice as determined by plating of gastric mucosal homogenates on horse blood plates and colony counting.
Fig. S4. VacA protects against allergic asthma when administered intraperitoneally or intragastrically. (A–E) Mice were i.p. injected weekly with 25 μg per dose of either recombinant GGT or purified wild-type or mutant (Δ6–27) VacA starting on day 7 of age until the second sensitization as described in Fig. 4 E–H (a subset of the mice of Fig. 4 E–H is shown here). (A and B) IL-13 and IL-5 secretion, as assessed by ELISA, of single cell lung preparations restimulated with ovalbumin. (C and D) Frequencies of CD103+ and CD11b+ cells among all CD11c+MHCII+B220−cDCs infiltrating the lungs of the indicated groups of mice. (E) Ratios of CD11b+ and CD103+ cDCs as calculated per mouse. (F and G) Purified wild-type VacA was either administered intraperitoneally (i.p.) or intragastrically (p.o.) as indicated. Mice received either 5 μg or 20 μg of VacA (as indicated), either from age day 7 onwards until 2 wk before challenge (as indicated by subscript “a”) or three doses only (delivered in weeks 1, 2, and 3 of life, denoted by subscript “b”) or as adults (denoted by subscript “c”). Two doses of IL-10R blocking antibody were administered during challenge where indicated. (F and G) Total cells and eosinophils contained in 1 mL of BALF. (H and I) Tissue inflammation and goblet cell metaplasia. Three doses of VacA delivered before weaning were insufficient to provide full protection (“b”). Treatment of adults was almost as protective as neonatal-onset treatment (compare “c” and “a”; note that the total cell count in the adult-treated group is inconsistent with the other three readouts for reasons that are not clear); the 5-μg and 20-μg doses provided similar levels of protection; blocking IL-10 signaling abrogated protection.